HIV-free survival at 12 – 24 months in breastfed infants of HIV-infected women on ART: a systematic review

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BACKGROUND

The 2010 WHO infant feeding guidelines in the context of HIV infection recommend exclusive breastfeeding (EBF) for six months followed by complementary feeding and continued breastfeeding up to one year of age, under the cover of antiretroviral treatment (ART) to either the mother or the infant [1]. However, when these recommendations were drawn up there was limited information about the risk of HIV transmission postnatally in women who were on ART during pregnancy and continued after delivery, or where the infant received ART prophylaxis, to prevent mother-to-child transmission (PMTCT). In the past five years, further evidence has become available from studies and programmes where PMTCT postnatally was achieved through maternal ART or infant ARV prophylaxis. The estimated risk of transmission and death (HIV-free survival) in the infants in such programmes remains to be clarified. It is also unclear is whether there continues to be an increased risk associated with mixed feeding compared with exclusive breastfeeding, which was seen in the pre-ART era, or whether the risk of transmission is reduced by effective ARV treatment to low levels irrespective of infant feeding modality.

WHO convened a Guideline Meeting in October 2015 to review the 2010 WHO guidelines on HIV and Infant Feeding in light of the expanding use of ART in pregnant and breastfeeding women. One question addressed in this meeting was: What is the relationship between infant feeding practices in the context of maternal ART, in particular in light of the duration of breastfeeding, and HIV-free survival in the infant?

We present the results from a systematic review and GRADE Evidence summary tables in preparation for this WHO guideline meeting. This first systematic review addresses the question of HIV-free survival at 12, 18 or 24 months in infants born to women who were on ART by infant feeding modality. We also present data on HIV transmission overall and by six months of age, and HIV-free survival by feeding modality. A second review presented separately summarises the evidence relating to breastfeeding modality in the first six months of life, with HIV transmission at six and 12 months as outcome.

METHODS

INCLUSION CRITERIA

Types of studies. The review considered both experimental and observational studies eg. randomised control studies, cohort studies, and longitudinal studies, and included HIV positive mothers receiving antiretroviral therapy and their breastfed children. Infants may also have received prophylactic ARVs as per WHO 2010 guidelines.

Types of participants. HIV positive mothers receiving combined antiretroviral therapy (ART) and their breastfed children.

Types of exposures. HIV antiretroviral therapy (and duration) and breastfeeding (and duration).

Outcome Measures. The outcome measures were HIV-free survival and HIV transmission between birth and 24 months of age.

SEARCH STRATEGY

SB searched English literature from multiple electronic databases including PubMed, MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, Web of Science, and CINAHL for articles with a time limit of 2005 to 2015 (Table 1). The search words in PubMed are shown in Table 2 and more detailed in Appendix 1.

SB did the initial search of literature in discussion with LC and MLN. The search terms were adapted for other databases. Reference lists from relevant studies, grey literature and conference abstracts available online from the following conferences were also searched: the International IAS AIDS Conference in Melbourne 2014 and the 2013-2015 Conferences on Retroviruses and Opportunistic Infections (CROI). SB further searched the reference lists of articles identified from the search of databases and conference abstracts. Also, in some cases where relevant information was not available in the publication, authors were contacted for specific additional information regarding infant feeding modality (data collection and support). Seven questions were asked of 10 first authors, additional information provided is included in the second systematic review which focussed on HIV transmission and infant feeding modality in the first six months of life.

Table 1. Electronic databases and conferences searched

Source	A: Identified	B: Selected by abstract[b]	C: Selected for full screening[c]	D: Included	D / B (%)
MEDLINE	467	125	46	16	12.8%
Web of Science	822	30[a]	8	1	3.3%
Conferences	6	1	0	0	0
Bibliography[d]			6	1	-
TOTAL	**1295**	**156**	**60**	**18**	

a Excluded duplicates
b Abstracts were rejected if mothers were not on ART or HIV-free-survival/transmission/mortality rates were not provided; where there was any detail regarding population and outcome, the paper was selected for full screening
c Full texts were rejected if outcome was not provided or when it was not possible to identify outcome by ART or feeding status.
d These papers were identified during full text screening from the references; 5 of these were excluded after abstract review.

The search process identified 1295 citations, of which 1139 were excluded on the basis of being a duplicate, review, qualitative study or not evaluating transmission, mortality or HIV-free-survival. The abstracts of the remaining 156 studies were evaluated by SB and LC (Table 1), and 54 texts were selected for full screening. SB, LC and MLN undertook the full text screening. Six additional articles from the references were identified through the full text screening bringing the total to 60 (Table 1).

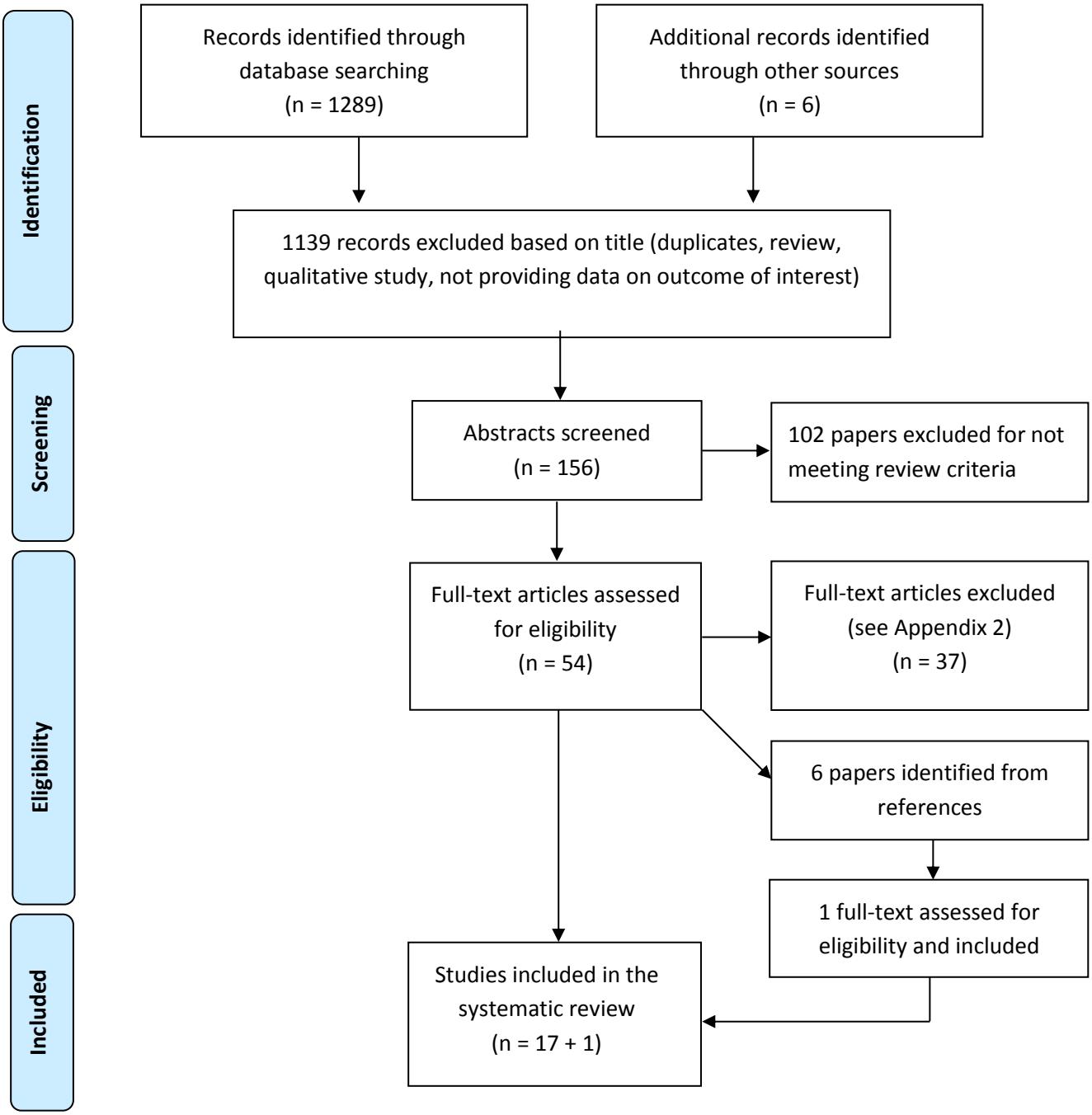
Table 2. Search Terms

Domain	Description	Search Terms
Antiretroviral	*Maternal*	Maternal, mother*
	Antiretroviral	Antiretroviral, antiretroviral therapy, ART, ARV, HAART
HIV free survival	*HIV free survival*	HIV free survival, HIV
	Mortality or transmission	Transm*, death, mortality
Feeding	*Feeding type*	Breastfeeding, breast*, postnatal, feeding
Publication Dates	*January 2005 to April 2015*	

Search terms were linked by OR or AND
** Used to include all the terms starting with the letters before the star*

Disagreement between reviewers occurred for 12 of the 60 fully screened papers, resulting in a Kappa coefficient of moderate agreement (0.57); further discussion between the three reviewers (SB, LC, MLN) resolved the issues. The majority of the excluded papers did not provide results for mothers on ART and breastfeeding at the same time but focused on different drug regimen or feeding (Appendix 2). The flow chart of the screening process is shown in Figure 1.

Figure 1. Flowchart of screening process



Eighteen studies were included in the analysis (Table 1, Figure 1); eight additional papers from selected studies provided additional information for the assessment of quality of studies and data collection. These included three studies from Kesho Bora [2-4], one from the HPTN trial [5], one from DREAM [6], two from MmaBana [7, 8] and one from the Kisumu Breastfeeding Study [9]. A full description of the included studies is given in Appendix 3. Of the 18 selected studies, one was performed in India and the remaining 17 were from African countries including: Botswana, Burkina Faso, Ivory Coast, Kenya, Malawi, Mozambique, Nigeria, Rwanda, South Africa, Tanzania, Uganda, Zambia and Zimbabwe. Five studies were conducted in rural areas [10-14] and the others in urban areas.

ASSESSMENT OF QUALITY OF STUDIES

Newcastle-Ottawa Scale

We developed a modified Newcastle-Ottawa Scale (NOS) to assess the quality of all studies included in the analysis [15, 16]; the criteria for assessment of the quality of studies are provided in Appendix 4, with the detailed NOS table in Appendix 5. Although some of the included studies were nested within RCTs, randomisation was not based on the intervention of interest (breastfeeding) and these studies were considered cohorts. Stars were awarded for each study based on selection of study participants and assessment of outcomes; assessment did not use all aspects of the NOS because the papers included in this research only assessed HIV-free survival in breastfed children whose mothers were on ART, and did not include a comparison group [15]. Each study could score a maximum of six stars on Selection and four on Outcome. The factors considered included the representativeness of the study population in terms of the underlying population of HIV-positive pregnant women accessing PMTCT programme, ascertainment of exposure to ART and breastfeeding, the basis of the ART in terms of HIV-disease progression, report of maternal adherence to ART and duration of breastfeeding. Ascertainment of outcome (HIV-free survival) included timing of assessment and whether the outcome was stratified by feeding, length of follow up and loss to follow up. A summary of the NOS is presented in the results section and a more detailed table is provided in appendix 5.

GRADE

The information obtained from the NOS was used to comment on the quality of the included studies in GRADE with respect to study limitations/risk of bias. We also considered

consistency of results, directness and publication bias. The GRADE Evidence Profiles are presented at the end of the results.

Synthesis of evidence

The studies covered different types of interventions and varied with regard to the outcomes of interest of indication, timing of initiation and duration of maternal ART, breastfeeding recommendations and practice. For example, one study compared HIV-free survival of children whose mothers were on ART and those whose mothers did not receive any ART postnatally [17], another compared HIV-free survival between children whose mothers received ART and those on short course ARVs [18], five studies compared HIV-free survival for children of mothers on different types of ART [2,19-21] and eight studies reported HIV-free survival of infants of women who were all on ART [10-14,18,22-24]. However, the latter eight studies differed in the duration of exclusive breastfeeding, which ranged from three to six months. With respect to timing of ART initiation for mothers, some studies reported an early start at 14- 15 weeks of pregnancy [23,25], while in the remaining studies ART was initiated only between 24 and 36 weeks of pregnancy. A detailed description of information provided by each study is given in Appendix 3.

We present the evidence using a narrative synthesis, in addition to a pooled estimate with a heterogeneity score based on a random effects meta-analysis in Stata. Random effects meta-analysis is recommended for use in the analysis of studies that were conducted differently [26]. The pooled estimate therefore represents the average estimate of HIV-free survival across the studies included in the analysis. We summarised the information in graphs depicting HIV-free survival rates by duration of maternal ART where possible and additionally presented HIV transmission rates for studies which provided transmission rates at six months and at the end of follow-up. For most cases confidence intervals for estimates of HIV-free survival and/or HIV transmission were given; where no confidence interval was available from the paper, a confidence interval was calculated based on the number of events and those at risk using the formula described by Eayres and shown in Appendix 6 [27]. To enable appropriate comparison of HIV-free survival between exclusively breastfed and formula-fed children we computed confidence intervals of the difference in the proportion of HIV-free survival between the two groups.

RESULTS

We identified 18 cohort studies, seven of which were nested within randomised clinical trials (Appendix 3). Most studies were a follow-up of mothers receiving antiretroviral therapy (ART) for PMTCT [10, 12, 13, 18, 22-25] with mothers advised to exclusively breastfeed for six months with rapid weaning thereafter, in line with the prevailing WHO recommendations at the time. Where women initiated ART for PMTCT, ART would have been stopped at cessation of breastfeeding around six months postpartum, also in line with the WHO recommendations at the time; seven studies offered lifelong ART irrespective of CD4 count and supported breastfeeding for 12 months [10,18,20,22,25,28,29]. Thomas *et al.* (2011) presented findings of a clinical trial evaluating two ART regimen, and is included in this review as a cohort with all women on ART postnatally. Thakwalakwa *et al.* (2014) randomised mothers/infants postpartum to two different types of complementary foods after weaning; all mothers received ART lifelong including throughout the breastfeeding period. Tonwe-Gold et al. (2007) presented findings of a cohort of women who received ART for life if they were ART-eligible as per WHO guidelines at the time or who received short-course ART for PMTCT only; data are included accordingly and relate to the women on ART only.

The seven cohorts nested within clinical trials aimed to evaluate the use of ART during pregnancy and postnatally in reducing MTCT and infant deaths [2,10,17,19-21,30]. Cournil *et al.* (2015) in the Kesho Bora study compared ART throughout six months of breastfeeding with short duration of peri-partum ARV, and presented HIV-free survival in breastfed children and in children given replacement feeding from birth. Jamieson *et al.* (2012) and Coovadia *et al.* (2012) compared rates and risk in children receiving prolonged nevirapine prophylaxis or not, with mothers on different types of ART treatment, but provided separate estimates on HIV-free survival for the group of mothers on ART for at least six months.

Quality of included Studies

Table 3 presents findings of the assessment of the quality of the studies based on the modified Newcastle-Ottawa Scale (Appendix 4). The study by Ngoma et *al.* (2015) had the highest quality in terms of Selection (6 stars), followed by Sagay *et al.* (2015), Cohan *et al.* (2015), Jamieson et al (2012), Thomas et al (2011) and Peltier et al (2009), all with four stars. Alvarez-Uria *et al.* (2012) and Tonwe-Gold *et al.* (2007) scored highest on quality of outcome assessment, with four stars. The detailed NOS table is provided in Appendix 5.

Table 3. Modified Newcastle-Ottawa for assessment of HIV-free Survival in breastfed infants whose mothers were on ART

Author	Country	Selection	Outcome
Ngoma et al, 2015	Zambia	******	**
Sagay et al, 2015	Nigeria	****	**
Cournil et al, 2015	Burkina Faso, Kenia and South Africa	**	***
Cohan et al, 2015	Uganda	****	**
Thakwalakwa et al, 2014	Malawi	**	**
Okafor et al, 2014	Nigeria	***	*
Giuliano et al, 2013	Malawi	**	***
Shapiro et al, 2013	Botswana	***	**
Coovadia et al, 2012	South Africa, Tanzania, Uganda and Zimbabwe	**	*
Jamieson et al, 2012	Malawi	****	**
Alvarez-Uria et al, 2012	India	**	****
Thomas et al, 2011	Kenya	****	***
Thistle et al, 2011	Zimbabwe	**	**
Homsy, 2010	Uganda	***	**
Peltier, 2009	Rwanda	****	***
Marazzi, 2009	Mozambique	**	***
Kilewo, 2009	Tanzania	***	***
Tonwe-Gold, 2007	Cote d'Ivoire	***	****

The majority of studies did not provide details regarding type of feeding, and assumed most mothers exclusively breastfed up to five or six months as recommended. Estimates of HIV-free survival by type of feeding could only be obtained from studies by Cournil *et al.* 2015, Alvarez-Uria *et al.*, 2012, Homsy *et al.*, 2009 and Peltier *et al.*, 2009 whilst Tonwe-Gold *et al.* 2007 presented information on HIV transmission by feeding type. Cournil *et al.* 2015 compared the rates in children who were either formula-fed from birth, breastfed for less than three months or for three months or longer. Three studies compared transmission or death between breastfed and formula-fed infants (Alvarez-Uria *et al.*, 2012; Peltier *et al.*, 2009; Homsy *et al.* 2009). Homsy *et al.* 2009 also provided separate results on HIV-free survival for children who were mixed-fed and those who weaned early.

Studies reported different ways of measuring HIV-free survival (Appendix 3). Eight studies provided HIV-free survival or rates of transmission and mortality from birth [12, 13, 18-21, 24, 28, 29]. Other studies excluded deaths and HIV transmission in the first days or weeks of life and provided only postnatal rates. For example Thakwalakwa *et al.* (2014) and Coovadia *et al.* (2012) excluded endpoints before six weeks; Giuliano *et al.* (2013) and Peltier *et al.*

(2009) excluded the first 24 hours; Jamieson *et al* (2013) and Cournil *et al.* (2015) excluded endpoints before two weeks, Thomas *et al.* (2011) excluded the first seven days, Alvarez-Uria *et al.* (2012) the first eight weeks and Marazzi *et al.* (2009) the first month of life. It was not clear in the study by Okafor *et al.* 2014 what criteria was used in the calculation of HIV-free survival estimate.

HIV-free Survival Estimates

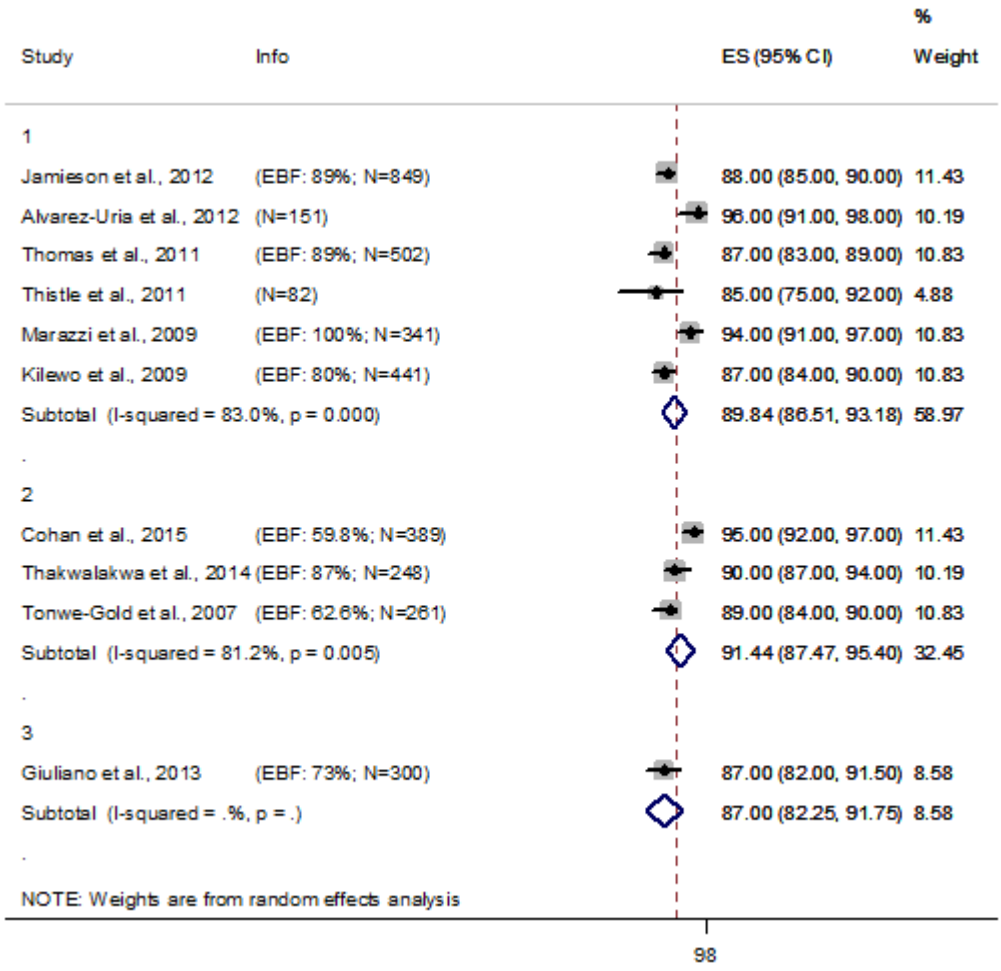
At age 12 months

Estimates of 12-month HIV-free survival, with confidence intervals, for breastfed infants by duration of maternal ART were obtained from ten studies (Figure 2). In six studies (Group 1 in Figure 2) HIV-free survival was reported for infants whose mothers were on ART up to six months postnatally, with estimates ranging from 85% (95% CI 75.0%, 92%) in Thistle et al. to 96% (95% CI 91%, 98%) in Alvarez-Uria et al.. The pooled estimated HIV-free survival was 89.8% (95% CI 86.5%, 93.2%) with considerable heterogeneity (I^2=83%).

In three studies, HIV-free survival was estimated amongst infants whose mothers were on lifelong ART (Group 2 in Figure 2) with estimated HIV-free survival ranging from 89% (95% CI 84%, 90%) in Tonwe-Gold et al. to 95% (95% CI 92%, 97%) in Cohan et al.. The pooled estimate was 91.4% (95% CI 87.5%, 95.4%) and heterogeneity was high (I^2=81.2%).

Giuliano et al. (Group 3 in Figure 2) with an estimate of 87.0% (95% CI 82.3%,91.8%) was based on a mixed group of infants with respect to mother's ART, some mothers were on ART up to six months postnatally whilst others were on life long ART.

Figure 2. 12-Month HIV-free Survival:
Group 1: Mothers on ART up to six months postnatally,
Group 2: Mothers on Lifelong ART,
Group 3: Mixture of Lifelong ART and ART up to six months postnatally



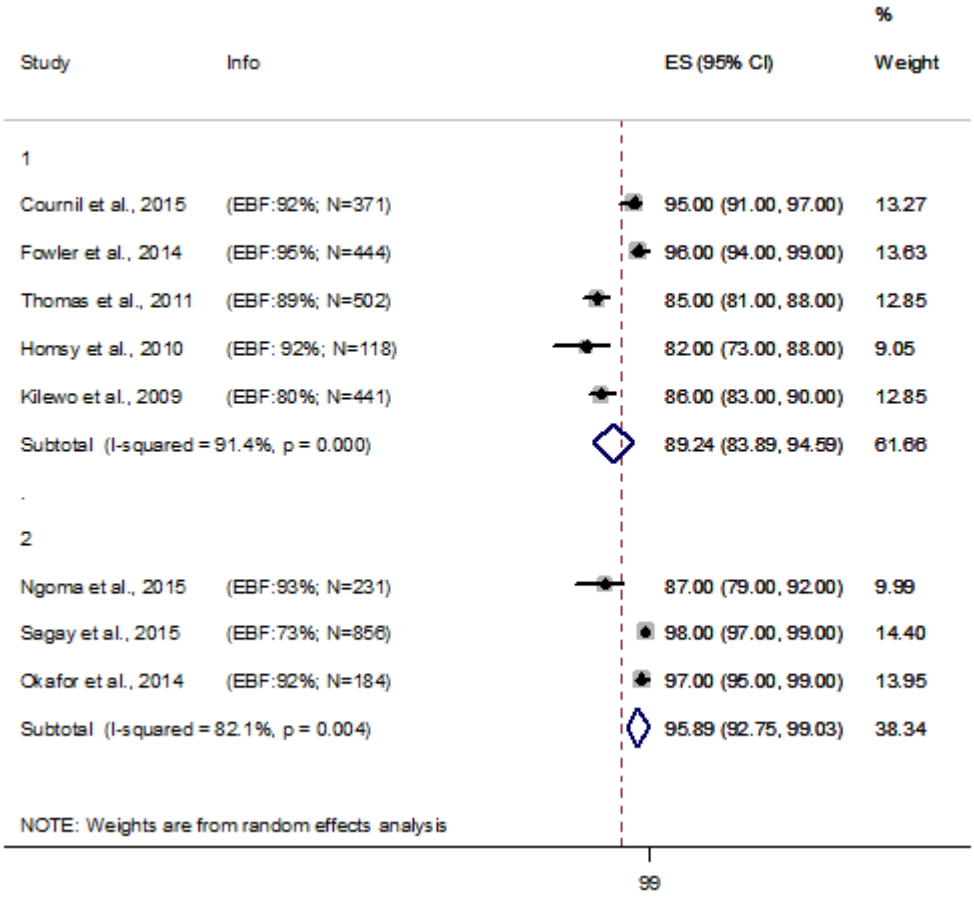
At age 18 months

Eight studies provided estimates for 18-month HIV-free survival and these are presented in Figure 3, by duration of maternal ART. In the six studies that reported HIV-free survival for infants of women who were on ART up to six months postnatally (Group 1 in Figure 3), HIV-free survival ranged from 82% (95% CI 73%, 88%) in Homsy et al. 2010 to 96% (95% CI 94%, 99%) in Fowler et al. 2014. The pooled estimate of HIV-free survival for this group was 89.2% (95% CI 83.9%, 94.6%) with substantial heterogeneity (I^2= 91.4%).

In studies where women were on lifelong ART (Group 2 in Figure 3) HIV-free survival estimates ranged from 87% (95% CI 79%,92%) in Ngoma et al. 2015 to 97% (95% CI 95%, 99%) in Okafor et al. 2014. The pooled estimated of HIV-free survival for this group was 95.9% (95% CI 92.8%, 99.3%) also with substantial heterogeneity (I^2= 82.1%).

Figure 3. 18-Month HIV-free survival in children whose mothers breastfed and were on ART,

Group 1: Mothers on ART up to six months postnatally,
Group 2: Mothers on lifelong ART

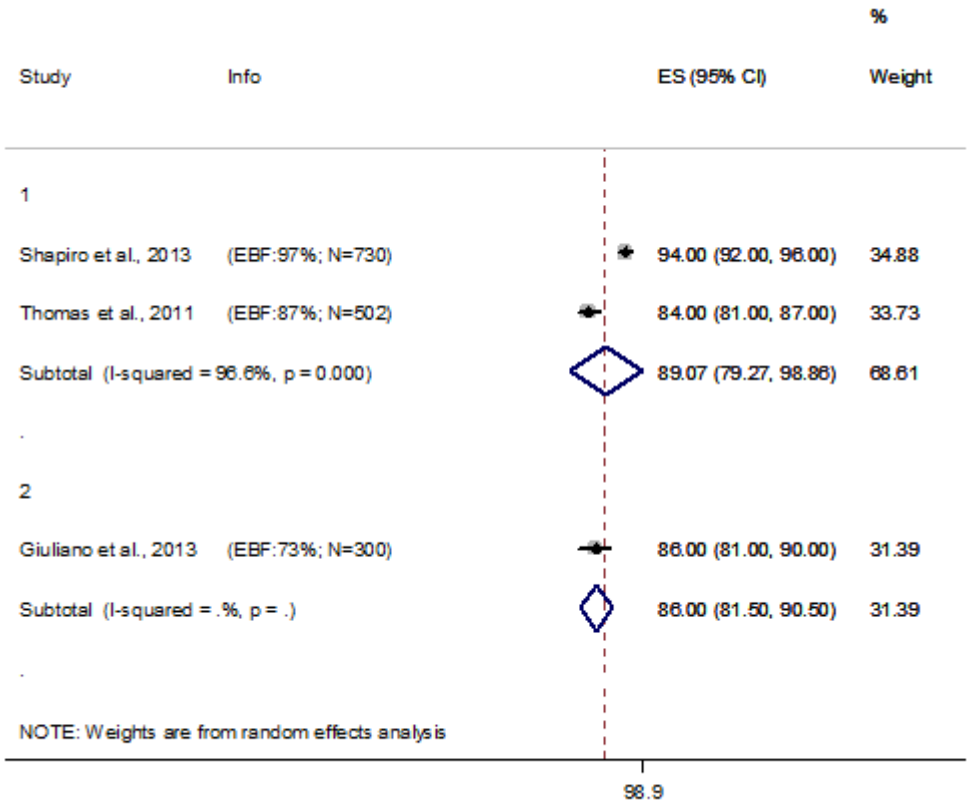


<u>At age 24 months</u>

Twenty-four-month HV-free survival estimates from three studies are shown in Figure 4. The estimates in the two studies that reported HIV-free survival amongst children whose mothers were on ART upto six months postnatally (Group 1 in Figure 4) were 84.3% (95% CI 80.6, 87.3%) in Thomas et al. and 94.0% (95% CI 92.1%, 95.9%) in Shapiro et al. The pooled estimate was 89.1% (95% CI 79.3%, 98.9%) and heterogeneity was extensive (I^2= 96.6%).

In Giuliano et al. (Group 2 in Figure 4) estimated HIV-free survival was 85.8% (95% CI, 81.4%, 90.1%), based on a mixed group of infants with respect to mother's ART, some were on ART up to six months postnatally whilst others were lifelong ART.

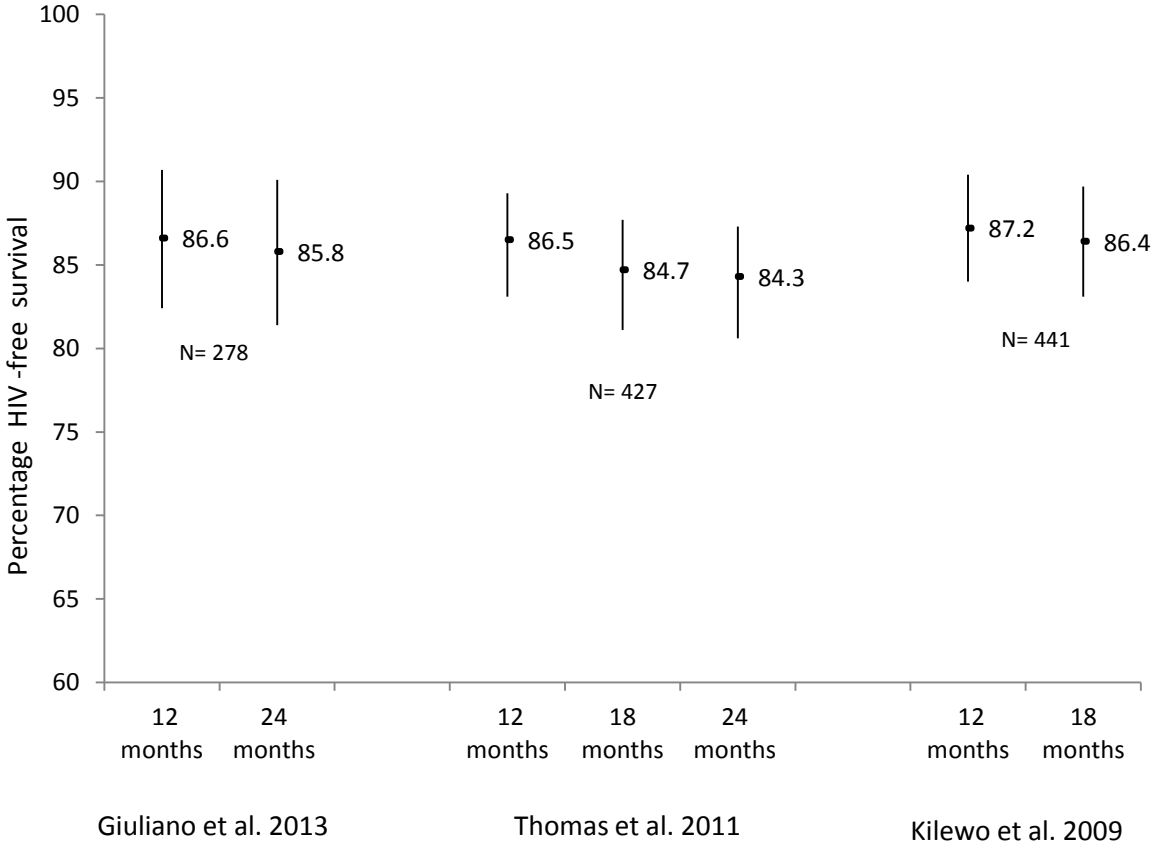
Figure 4. 24-Month HIV-free survival in children whose mothers breastfed and were on ART.
Group 1: Mothers on ART up to six months postnatally,
Group 2: Mixture of mothers, some on lifelong ART and others on ART up to six months postnatally



HIV-free survival at 12, 18 and 24 months

Three studies provided estimates of the percentage of HIV-free survival at 12 months, 18 months and 24 months allowing a comparison of HIV-free survival between 12 and 24 months (Figure 5). In all three studies, HIV-free survival at 18 months and/or 24 months was slightly lower than the estimate at 12 months, but not statistically significantly so. However, it should be noted that the sample size reported for each study was related to the start of the period of observation and it was not possible to obtain sample sizes to calculate HIV-free survival in different age intervals.

Figure 5. HIV-free survival at 12, 18 and 24 months, with 95% confidence intervals, in children who were breastfed and whose mothers were on ART

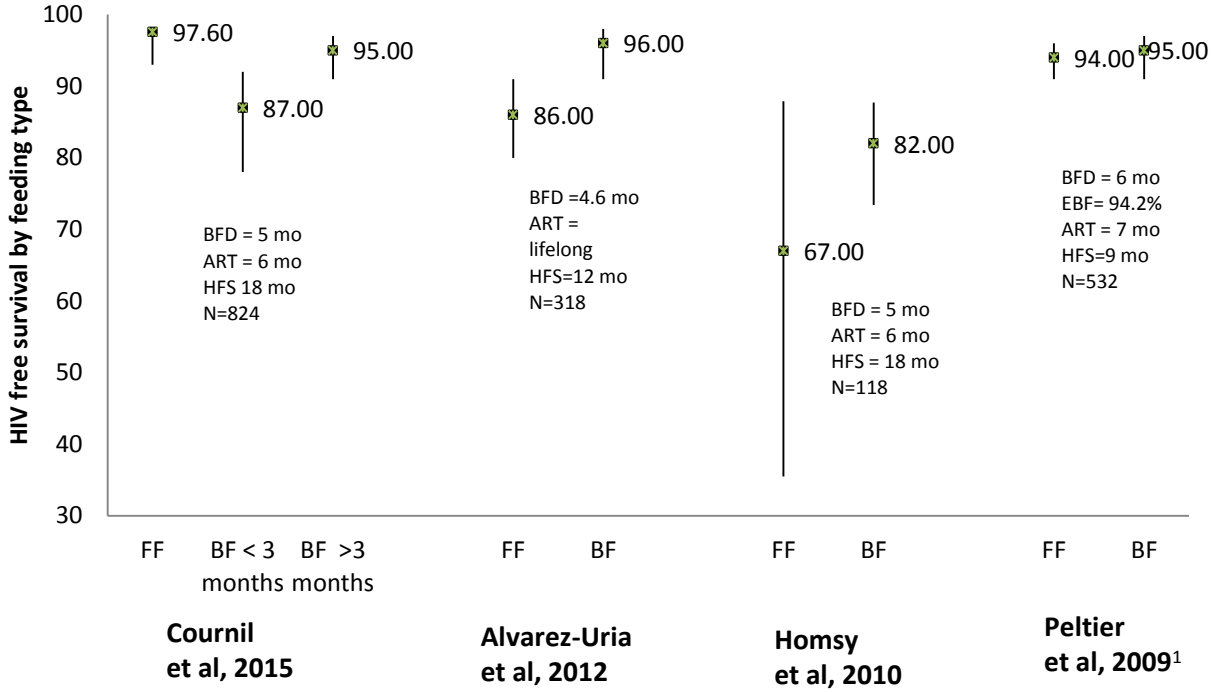


By infant feeding modality

HIV-free survival by feeding type from four studies is shown in Figures 6 and 7. In the study by Cournil et al. estimated 18-month HIV-free survival was significantly higher among formula-fed than infants breastfed for less than three months (Figures 6 and 7); the difference between formula-fed infants and those breastfed for more than three months was not significant. In the studies by Alvarez-Uria et al., reporting estimates at 18 months, and Peltier et al. [1] at nine months, HIV-free survival was significantly higher amongst breastfed than formula-fed children, but the difference was not statistically significant in Homsy et al at 12 months (Figure 7).

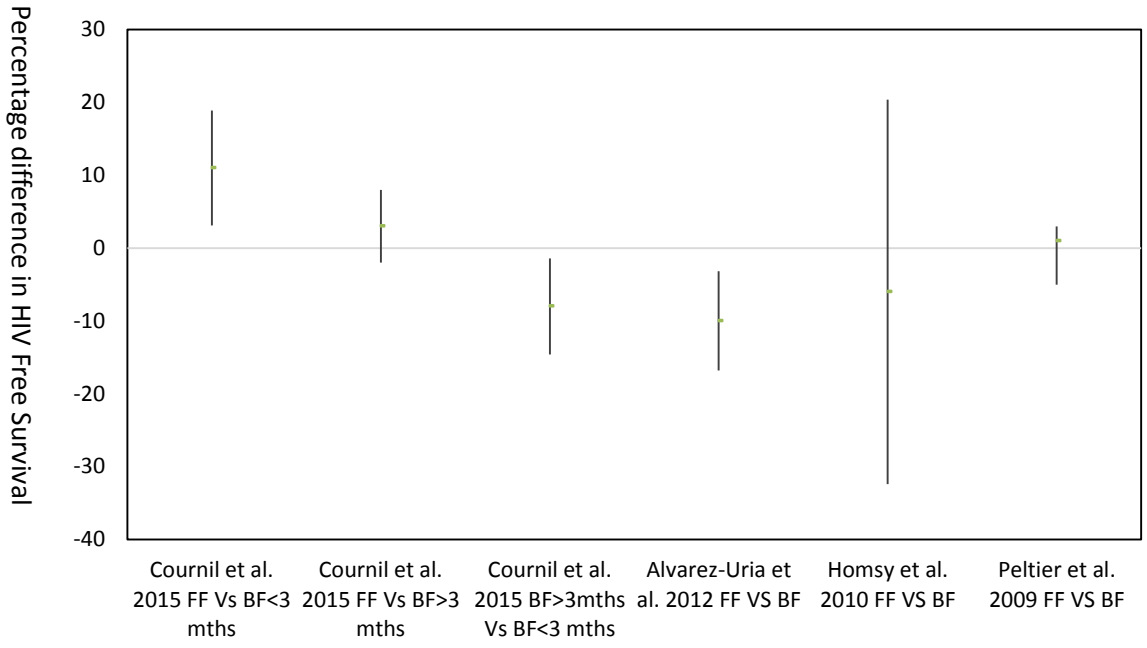
[1] The study by Peltier et al. 2009 presented HIV Free Survival at 9 months and therefore not included the previous analyses

Figure 6. HIV-free survival by feeding modality in four studies



[1] The study by Peltier et al. 2009 presented HIV Free Survival at 9 months and therefore not included the previous analyses

Figure 7. Percentage difference in HIV-free survival by feeding type



Notes: Estimates of HIV-free-survival considered mothers on HAART according to type of feeding. Mothers received HAART for 6 months or during breastfeeding. Differences among the studies are described below.
FF: Formula fed, BF: Breast fed

1) Cournil *et al.,* 2015 excluded mothers in clinical stage 4 or with CD4 <200 cells/mm^3. HIV-free survival excluded endpoints during the first 2 weeks, and was measured at 18 months.
2) Alvarez Uria *et al.,* 2012: HIV free survival was obtained after 8 weeks, and measured at 12 months.
3) Homsy *et al.,* 2010: Mothers received ART when CD4 cell counts ⩽250 cells/μL or when with WHO stage III or IV disease. There was no transmission in the study, but 4 children who died had not been tested. HIV-free survival was not provided according to feeding modality, but numbers of deaths by feeding were provided. Among 118 children, only 9 were formula fed, of whom 3 died;2 received mixed feeding, and both died.
4) Peltier *et al.,* 2009: Mothers with CD4 cell counts ≤350 cells/mm^3 remained on ART. HIV free survival was measured at 9 months and endpoints were considered from 24 hours.

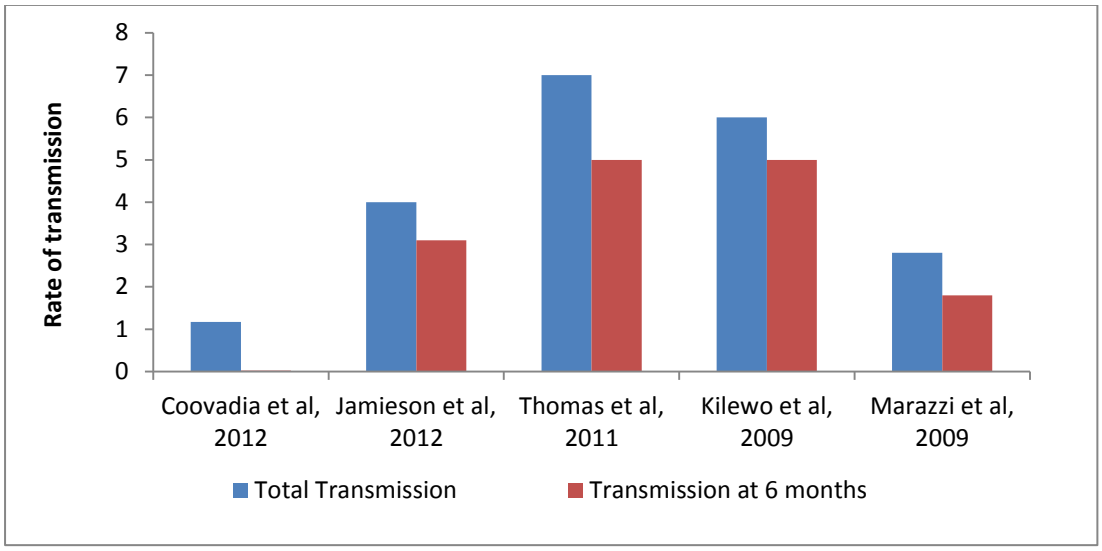
Transmission

Eight studies provided estimates of death and transmission overall and after six months of age, when the infant would have been weaned and mothers were no longer receiving ART. In Ngoma et al. there were no HIV transmissions between six weeks and six months, three infections occurred peripartum, and six after six months. In the study by Giuliano et al there were eight infections overall, four occurred before weaning at six months and four after

weaning; two of the four mothers that transmitted HIV after weaning were still receiving ART. Similarly, in Kilewo et al. the numbers before and after were the same: 4 of 8.

In the other studies there were fewer infections after six months of age than before: Jamieson et al., 9 of 30, Thomas et al. 8 of 32, Cournil et al. 4 of 9; Marazzi et al. 2 of 8; and in Sagay et al. 2 of 6; Figure 8 shows HIV transmission rates overall and by six months of age for the five studies which provided this information.

Figure 8. HIV transmission rate overall and at age 6 months (at breastfeeding and ART cessation), by study



Mortality

The pattern of infant deaths occurring before and after weaning varied across studies. The study by Jamieson et al. reported the same number of deaths before and after weaning (9 of 18 deaths before weaning); Tonwe-Gold et al. reported a lower number of deaths after weaning (1 in 9) than before weaning. Thomas et al. and Shapiro et al. reported higher numbers of deaths after the weaning period, (31 of 49 and 22 of 37 respectively). Shapiro et al. showed that the death rate within 3 months of weaning was significantly higher than during breastfeeding (RR=3.7; 1.3-12.0; p=0.007), and the same was found in comparing those who were weaned before three months of age and ≥3 months (RR=7.5; 3.2-18.4; p<0.001). In Kilewo et al. 21 of 31 deaths occurred after breastfeeding cessation; in Marazzi et al. seven of 11 deaths and Ngoma et al. eight of 20 deaths occurred after weaning. Courrnil et al. did not report deaths after 6 months, but reported a higher risk of dying by six months

of age in children who stopped breastfeeding before three months of age compared to those who stopped ≥3 months (HR: 3.94-1.27-12.27).

GRADE PROFILE

An evaluation of the quality of the studies considered in this analysis is given in Table 4. The assessment of quality was based on study limitations/risk of bias as per the evidence from the Newcastle-Ottawa Scale (Table 3, Appendix 5). We also considered inconsistency, indirectness and publication bias. Initially, all studies were scored low quality due to being observational and were downgraded for indirectness because their research areas were not directly in line with the PICO question. Where a pooled analysis was undertaken and a pooled estimate provided, studies were further downgraded for inconsistency. In all groups of studies there was at least one study with a risk of bias pertaining to lack of detailed information on feeding leading to further downgrading. In one study, the criteria for assessing HIV-free survival was not clear (Okafor et al. 2014) and this contributed to the downgrading of the quality of the studies grouped together with this study. Only one study (Giuliano et al.) was not downgraded for risk of bias and inconsistency.

Table 4 Grade Evidence Profiles

Question: HIV-free survival in Breastfed Infants of mothers on ART [1] [2]

Setting: India, Botswana, Burkina Faso, Ivory Coast, Kenya, Malawi, Mozambique, Nigeria, Rwanda, South Africa, Tanzania, Uganda, Zambia and Zimbabwe

Quality assessment							№ of patients	Effect	Quality	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Total number of breastfed infants of mothers on ART**	**Percentage of HIV Free Survival (95% CI)**		
HIV Free Survival at 12 Months: Mothers on ART up 6 months postnatal										
6	observational studies [3]	serious [4]	serious [5]	serious [6]	not serious	none	2533	89.8% (86.5%, 93.2%)	⊕◯◯◯ VERY LOW [4] [5] [6]	CRITICAL
HIV Free Survival at 12 Months: Mothers on life long ART										
3	observational studies [7]	serious [8]	serious [9]	serious [10]	not serious	none	1198	91.4% (87.5%,95.4%)	⊕◯◯◯ VERY LOW [8] [9] [10]	CRITICAL
HIV Free Survival at 12 Months: Mixture of mothers some on life long ART and others on ART up to 6 months postnatal										
1	observational studies [11]	not serious	not serious	serious [12]	not serious	none	300	87.0% (82.3%,91.8%)	⊕◯◯◯ VERY LOW [12]	CRITICAL
HIV Free Survival at 18 Months: Mothers on ART up to 6 months postnatal										

Quality assessment							№ of patients	Effect	Quality	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Total number of breastfed infants of mothers on ART	Percentage of HIV Free Survival (95% CI)		
5	observational studies [13]	serious [14]	serious [15]	serious [16]	not serious	none	2905	89.1% (79.3%, 98.9%)	⊕◯◯◯ VERY LOW [14 15 16]	CRITICAL
HIV Free Survival at 18 Months: Mothers on life long ART										
3	observational studies [17]	very serious [18]	serious [19]	serious [20]	not serious	none	1271	95.9% (92.8%, 99.3%)	⊕◯◯◯ VERY LOW [18 19 20]	CRITICAL
HIV Free Survival at 24 Months: Mothers on ART up 6 months postnatal										
2	observational studies [21]	serious [22]	serious [23]	serious [24]	not serious	none	1232	89.1% (79.3%, 98.9%)	⊕◯◯◯ VERY LOW [22 23 24]	CRITICAL
HIV Free Survival at 24 months: Mixture of mothers, some on life long ART and others on ART up 6 months postnatal										
1	observational studies [11]	not serious	not serious	serious [12]	not serious	none	300	85.8% (81.4%, 90.1%)	⊕◯◯◯ VERY LOW [12]	CRITICAL

MD – mean difference, RR – relative risk

1. There was no comparison in line with the PICO question. In all studies the recommendation was exclusive breastfeeding in the first six months and the majority of mothers breastfed their children.
2. The estimate of HIV-free Survival is a total for all infants in the studies where the majority were breastfed.

3. Four studies; Alvarez-Uria et al 2012, Thistle et al. 2011, Marazzi et al. 2009 and Kilewo et al. 2009 were cohort studies, whilst the studies by Jamieson et al. 2012 and Thomas et al. 2011 were cohorts embedded in randomised control trials.
4. Risk of bias: We downgraded once due to potential selection bias for lack of detailed feeding history in Marazzi et al. 2009, Thistle et al. 2011 and Alvarez-Uria et al. 2012 and 20% loss to follow up in Jamieson et al. 2012 and 18.3% loss to follow up in Thistle et al. 2011.
5. Inconsistency: We downgraded once due to substantial heterogeneity in the pooled estimate, I squared= 83%. HIV Free Survival ranged from 85% (95% CI 75%, 92%) Thistle et al. 2011 to 96 %(95% CI 91% to 98%) Alvarez-Uria et al, 2012.
6. Indirectness: We downgraded once because studies' research questions were not in line with the PICO question and they covered different types of co-interventions. In Thistle et al 2011, Marazzi et al. 2009 and Kilewo et al. 2009 the only intervention was ART, Jamieson et al. 2012 compared HIV Free Survival in children whose mothers were on ART, infants on NVP and a control group and the three groups were further divided into those who were on a maternal nutrition supplement and those that weren't. In Alvarez-Uria et al. 2012 all women were on ART but newborns were also given prophylaxis. In Thomas et al. 2011 all mothers were on ART but all infants received a single dose of NVP within 72 hours and TMP/SMX from 6 weeks. Studies also varied with regard to the outcomes of interest of indication, timing of initiation of maternal ART and breastfeeding recommendations and practice. There were no comparative studies.
7. The study by Tonwe-Gold et al. 2007 was a cohort study whilst the studies by Cohan et al. 2015 and Thakwalakwa et al. were cohort studies embedded within randomised control trials.
8. Risk of bias: We downgraded once due to the potential selection bias for lack of detailed feeding history in Tonwe-Gold et al. 2007 and Thakwalakwa et al. 2011.
9. Inconsistency: We downgraded once due to substantial heterogeneity in the pooled estimate, I squared=81.2%. HIV Free Survival estimates ranged from 89% (95% CI 83%, 95%) Tonwe-Gold et al. 2007 to 95 %(95% CI 92% to 97%) Cohan et al. 2015.
10. Indirectness: We downgraded once as the studies' questions were not in line with the PICO question and covered different types of co-interventions. In the study by Tonwe-Gold et al. 2007, all women were on ART and infants received sdNVP at 3 days of life and 1 week of ZDV syrup. In the study by Cohan et al. 2015, mothers were randomised to receive two different types of ART. One group received efavirenz whilst the other received lopinavir/rotonavir and all women received trimethoprim-sulfamethoxazole prophylaxis. In Thakwalakwa et al. all mothers were on ART but at 6 months infants were randomised to receive either milk powder or a ready to use food comprising of peanut paste, skimmed powder, sugar and vegetable oil. Studies also varied with regard to the outcomes of interest of indication, timing of initiation of maternal ART and breastfeeding recommendations and practice. There were no comparative studies
11. The study by Giuliano et al. 2013 was a retrospective cohort study. ART stopped at 6 months for those with CD4+ count greater than 350/mm^3 but continued in those with a CD4+ count less than 350/mm^3
12. Indirectness: We downgraded once since the study research question was not in line with the PICO question. The study assessed HIV Free Survival amongst breastfeeding infants whose mothers were on ART
13. The studies by Cournil et al. 2015, Covaadia et al. 2012, Thomas et al. 2011 were cohort studies embedded in randomised control trials whilst Homsy et al. 2010 and Kilewo et al. 2009, were cohort studies.
14. Risk of bias. We downgraded once due to potential selection bias for lack of feeding history in the study by Thomas et al. 2011.
15. Inconsistency: We downgraded once due to substantial heterogeneity in the pooled estimate, I squared=91.4%. HIV Free Survival ranged from 82 %(95% CI 73%, 88%) Homsy et al. 2010 to 96 %(95% CI 94%, 99%).
16. Indirectness: We downgraded once because the studies' research question was not in line with the PICO question and covered different types of co-interventions; In Cournil et al. 2015 women were randomised to receive triple ARV prophylaxis or short course ARV. In Coovadia et al. 2012, infants were randomised to receive either extended nevirapine prophylaxis or placebo until 6 months or until breastfeeding cessation. In Thomas et al. 2011 all mothers were on ART but all infants received a single dose of NVP within 72 hours and TMP/SMX from 6 weeks. In Homsy et al. all women were on ART and infants received a single dose of NVP within 72 hours of birth. The NVP was later supplemented with AZT syrup during the course of the study. Eligibility for ART in Homsy et al. 2010 was CD4 cell counts equal or les s than 250 cell/uL or WHO Stage 3 or 4. In Kilewo et al. 2009 all mothers were on ART and infants received ZDV+3TC for 1 week after birth. Studies also varied with regard to the outcomes of interest of indication, timing of initiation of maternal ART and breastfeeding recommendations and practice. There were no comparative studies.
17. The studies by Ngoma et al. 2015, Sagay et al. 2015 and Okafor et al. 2014 were cohort studies
18. Risk of Bias: We downgraded twice because Sagay et al. 2015 had a problem with lack of detailed feeding history and Okafor et al. 2014 did not provide a criteria of how they estimated HIV Free survival.
19. Inconsistency: We downgraded once because of substantial heterogeneity in the pooled estimate, I squared= 82%. HIV Free survival ranged from 87 %(95% CI 79%, 92%) Ngoma et al. 2015 to 98 %(95% 97%, 99%) Sagay et al. 2015.
20. Indirectness: We downgraded once because the studies' research questions were not in line with the PICO question and there were slight differences in the co-interventions. In Ngoma et al. 2015 all women were on ART, in Sagay et al. 2015 and Okafor et al. apart from all women being on ART, HIV exposed infants received NVP from birth up to 6 weeks.
21. The two studies by Thomas et al. 2011 and Shapiro et al. 2013 were cohorts embedded within randomised control trials
22. Risk of bias. We downgraded once for potential risk of selection bias due to lack of detailed feeding history in the study by Thomas et al. 2011

23. Inconsistency: We downgraded once due to substantial heterogeneity, I squared=97%. HIV free Survival was 84.35 (95% CI 81%, 87%) in the study by Thomas et al. 2010 and 94 %(95% CI 92%, 96%) in the study by Shapiro et al. 2013.
24. Indirectness: We downgraded once since the study research question was not in line with the PICO question. In Shapiro et al. women received different types of ART depending on CD4 cell count whilst in Thomas et al. 2011 apart from all mothers being on ART all infants received a single dose of NVP within 72 hours and TMP/SMX from 6 weeks.

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feeding to prevent HIV postnatal mother-to-child transmission in Rwanda. *AIDS (London, England)* 2009, **23**(18):2415-2423.
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Appendix 1 Search words for identification of studies

The search words in PubMed were: *(Antiretroviral therapy[Title/Abstract] OR Antiretroviral*[Title/Abstract] OR HAART[Title/Abstract] OR ART[Title/Abstract] OR ARV[Title/Abstract]) AND (HIV[Title/Abstract] OR Nevirapine[Title/Abstract]) AND (TRANSMISSION[TITLE/ABSTRACT] OR Postnatal*[Title/Abstract]) AND (Breastfeeding[Title/Abstract] OR Breast*[Title/Abstract] OR feeding[Title/Abstract] OR replacement f* OR mixed*) AND ("2005"[Publication Date] : "2015"[Publication Date])).*

Appendix 2 Excluded papers [2]with reason for exclusion, after full text screening

No.	Reference	Reason for Exclusion
1.	Anoje C, Aiyenigba B, Suzuki C, Badru T, Akpoigbe K, Odo M, et al. Reducing mother-to-child transmission of HIV: findings from an early infant diagnosis program in south-south region of Nigeria. BMC Public Health. 2012;12:184.	The study combines both groups of women on different types of ARV, and do not provide infant HIV free survival when mothers are breastfeeding and on ART.
2.	Becquet R, Bequet L, Ekouevi DK, Viho I, Sakarovitch C, Fassinou P, et al. Two-year morbidity-mortality and alternatives to prolonged breast-feeding among children born to HIV-infected mothers in Cote d'Ivoire. PLoS Med. 2007;4(1):e17	Mothers were not on ART, but on dual ARV with single dose NVP on labour.
3.	Becquet R, Ekouevi DK, Menan H, Amani-Bosse C, Bequet L, Viho I, et al. Early mixed feeding and breastfeeding beyond 6 months increase the risk of postnatal HIV transmission: ANRS 1201/1202 Ditrame Plus, Abidjan, Cote d'Ivoire. Preventive Medicine. 2008;47(1):27-33.	Mothers were not on ART, but on dual ARV with single dose NVP on labour.
4.	Binagwaho A, Pegurri E, Drobac PC, Mugwaneza P, Stulac SN, Wagner CM, et al. Prevention of mother-to-child transmission of HIV: cost-effectiveness of antiretroviral regimens and feeding options in Rwanda. PLoS One. 2013;8(2):e54180	Mothers are on short course ART, and does not provide HIV free survival. Focused on costs.
5.	Chi BH, Musonda P, Lembalemba MK, Chintu NT, Gartland MG, Mulenga SN, et al. Universal combination antiretroviral regimens to prevent mother-to-child transmission of HIV in rural Zambia: a two-round cross-sectional study. Bulletin of the World Health Organization. 2014;92(8):582-92.	Provides only total HIV free survival, not total number of mothers on ART or infection only by ART.
8.	Derebe G, Biadgilign S, Trivelli M, Hundessa G, Robi ZD, Gebre-Mariam M, et al. Determinant and outcome of early diagnosis of HIV infection among HIV-exposed infants in southwest Ethiopia. BMC research notes. 2014;7:309	No rates for breastfeeding and ART together were provided.
10.	Goga AE, Dinh TH, Jackson DJ, Lombard C, Delaney KP. First population-level effectiveness evaluation of a national programme to prevent HIV transmission from mother to child, South Africa. 2015;69(3):240-8	It is very early diagnosis (4-6 weeks).
11.	Gray GE, Urban M, Chersich MF, Bolton C, van Niekerk R, Violari A, et al. A randomized trial of two postexposure prophylaxis regimens to reduce mother-to-child HIV-1 transmission in infants of untreated mothers. AIDS (London, England). 2005;19(12):1289-97	Mothers were not on ART, but on dual ARV.
12.	Kagaayi J, Gray RH, Brahmbhatt H, Kigozi G, Nalugoda F, Wabwire-Mangen F, et al. Survival of infants born to HIV-positive mothers, by feeding modality, in Rakai, Uganda. PLoS One. 2008;3(12):e387	Mothers on different type of antiretroviral therapy. Not possible to identify HIV transmission and death by mothers on ART.
14.	Kouanda S, Tougri H, Cisse M, Simpore J, Pietra V, Doulougou B, et al. Impact of maternal HAART on the prevention of mother-to-child transmission of HIV: results of an 18-month follow-up study in Ouagadougou, Burkina Faso. AIDS care. 2010;22(7):843-50	Only 8 mothers on ART were breastfeeding on the first exam.
15.	Kuhn L, Aldrovandi GM, Sinkala M, Kankasa C, Semrau K, Kasonde P, et al. Differential effects of early weaning for HIV-free survival of children born to HIV-infected mothers by severity of maternal disease. PLoS One. 2009;4(6):e6059	Mothers received single-dose nevirapine.
16	Leroy V, Ekouevi DK, Becquet R, Viho I, Dequae-Merchadou L, Tonwe-Gold B, et al. 18-month effectiveness of short-course antiretroviral regimens combined with alternatives to breastfeeding to prevent HIV mother-to-child transmission. PLoS One. 2008;3(2):e1645	Mothers were not on ART, but on dual ARV with single dose NVP on labour.
17.	Magoni M, Bassani L, Okong P, Kituuka P, Germinario EP, Giuliano M, et al. Mode of infant feeding and HIV infection in children in a program for prevention of mother-to-child transmission in Uganda. AIDS (London, England). 2005;19(4):433-7	Mother receiving short course ART.
18.	Mandala J, Moyo T, Torpey K, Weaver M, Suzuki C, Dirks R, et al. Use of service data to inform pediatric HIV-free survival following prevention of mother-to-child transmission programs in rural Malawi. Bmc Public Health. 2012;12	Mothers on single dose NVP.
19.	Minniear TD, Girde S, Angira F, Mills LA, Zeh C, Peters PJ, et al. Outcomes in a cohort of women who discontinued maternal triple-antiretroviral regimens	Mothers discontinued ART after labour.

[2] Studies are based on published papers, which were screened based on the search criteria in Appendix 1. Some studies are additional outputs of larger studies that produced further papers and reports not considered in this study.

	initially used to prevent mother-to-child transmission during pregnancy and breastfeeding--Kenya, 2003-2009. PLoS One. 2014;9(4):e93556	
20.	Mwendo EM, Mtuy TB, Renju J, Rutherford GW, Nondi J, Sichalwe AW, et al. Effectiveness of prevention of mother-to-child HIV transmission programmes in Kilimanjaro region, northern Tanzania. Tropical medicine & international health : TM & IH. 2014;19(3):267-74	Almost 50% of losses on the first PCR test, and only 7 children completed 18 months follow-up.
21.	Nagot N, Kankasa C, Meda N, Hofmeyr J, Nikodem C, Tumwine JK, et al. Lopinavir/Ritonavir versus Lamivudine peri-exposure prophylaxis to prevent HIV-1 transmission by breastfeeding: the PROMISE-PEP trial Protocol ANRS 12174. BMC infectious diseases. 2012;12:246	Mothers included on the study were not eligible for ART.
22.	Nlend AEN, Ekani BB. Preliminary assessment of breastfeeding practices in HIV 1-infected mothers (prior to weaning) under the Djoungolo programme on the prevention of mother-to-child transmission of HIV. Journal of tropical pediatrics. 2010;56(6):436-9	The paper focus on breastfeeding, mastitis and transmission, and assessment of transmission done at 13 weeks.
23.	Nyandiko WM, Otieno-Nyunya B, Musick B, Bucher-Yiannoutsos S, Akhaabi P, Lane K, et al. Outcomes of HIV-exposed children in western Kenya: efficacy of prevention of mother to child transmission in a resource-constrained setting. Journal of acquired immune deficiency syndromes (1999). 2010;54(1):42-50	Not possible to extract transmission and death for ART and BF together.
24.	Omer SB. Twelve-month follow-up of Six Week Extended Dose Nevirapine randomized controlled trials: differential impact of extended-dose nevirapine on mother-to-child transmission and infant death by maternal CD4 cell count. AIDS (London, England). 2011;25(6):767-76	Most mothers were not on ART.
25.	Palombi L, Marazzi MC, Voetberg A, Magid NA. Treatment acceleration program and the experience of the DREAM program in prevention of mother-to-child transmission of HIV. AIDS (London, England). 2007;21 Suppl 4:S65-71	Same data from DREAM study as another selected study.
26.	Read JS. Prevention of mother-to-child transmission of HIV: antiretroviral strategies. Clinics in perinatology. 2010;37(4):765-76, viii	Very small number of mothers on ART.
27.	Seth A, Chandra J, Gupta R, Kumar P, Aggarwal V, Dutta A. Outcome of HIV exposed infants: experience of a regional pediatric center for HIV in North India. Indian J Pediatr. 2012;79(2):188-93	Very small number of mothers on ART, and not provided information if those mothers were breastfeeding or not.
28.	Shah M, Johns B, Abimiku Al, Walker DG. Cost-effectiveness of new WHO recommendations for prevention of mother-to-child transmission of HIV in a resource-limited setting. AIDS (London, England). 2011;25(8):1093-102.	Study based on models of % of adherence in Nigeria.
31.	Simpore J, Pietra V, Pignatelli S, Karou D, Nadembega WM, Ilboudo D, et al. Effective program against mother-to-child transmission of HIV at Saint Camille Medical Centre in Burkina Faso. Journal of medical virology. 2007;79(7):873-9	Very small number of breastfed children.
32.	Stringer JS, Stinson K, Tih PM, Giganti MJ, Ekouevi DK, Creek TL, et al. Measuring coverage in MNCH: population HIV-free survival among children under two years of age in four African countries. PLoS Med. 2013;10(5):e1001424	Only provide HIV free survival for mothers on ART or dual ARV together.
33.	Taha TE, Li Q, Hoover DR, Mipando L, Nkanaunena K, Thigpen MC, et al. Postexposure Prophylaxis of Breastfeeding HIV-Exposed Infants With Antiretroviral Drugs to Age 14 Weeks: Updated Efficacy Results of the PEPI-Malawi Trial. Jaids-Journal of Acquired Immune Deficiency Syndromes. 2011;57(4):319-25	Mothers not on ART, comparison among dual therapy
34.	Torpey K, Kabaso M, Weaver MA, Kasonde P, Mukonka V, Bweupe M, et al. Infant feeding options, other nonchemoprophylactic factors, and mother-to-child transmission of HIV in Zambia. Journal of the International Association of Physicians in AIDS Care (Chicago, Ill : 2002). 2012;11(1):26-33	No data for mother on ART and breastfeeding together
35.	Torpey K, Kasonde P, Kabaso M, Weaver MA, Bryan G, Mukonka V, et al. Reducing pediatric HIV infection: estimating mother-to-child transmission rates in a program setting in Zambia. Journal of acquired immune deficiency syndromes (1999). 2010;54(4):415-22	No data for mother on ART and breastfeeding together
36.	van Lettow M, Bedell R, Landes M, Gawa L, Gatto S, Mayuni I, et al. Uptake and outcomes of a prevention-of mother-to-child transmission (PMTCT) program in Zomba district, Malawi. BMC Public Health. 2011;11:426	Very small number of mothers on ART

Appendix 3 Included Studies: Descriptive information of studies providing information on breastfeeding and ART

First Author/Study	Place of study	Randomised for		Feeding	Beginning/ end ART	Breast-feeding duration	Time evaluation	N	Extracted information	
		ARV	Other						HFS	Included
Cohorts embedded on RCTs										
Kesho Bora Study (Cournil, 2015-2013; de Vicenzi, 2011-2010	Burkina Faso, Kenya and South Africa	HAARTsc ARV†		BF<3 mo, BF≥ 3mo, RF	34wk/ cessation BF	6 mo	12, 18 mo	371	Given	Mortality and Transmission from 2 weeks
HPTN046 trial (Fowler, 2014; Coovadia, 2012)	South Africa, Tanzania, Uganda and Zimbabwe	HAART no ARV	Infant NVP or not	All BF	From first antenatal visit/ 6 mo	6 mo	18 mo	1527	Given	Transmission from 6 weeks
Mma Bana Study (Shapiro et al, 2013,2010 and 2009)	Botswana	2 types of HAART†		Majority BF	26 or 34 wks/ 6 mo‡	6 mo	24 mo	730	Given	All transmission and mortality
Jamieson, 2012	Antenatal clinics in Malawi	HAART† or infant NVP	Nutritional intervention	Majority BF	30wks or less/ 6 mo	6 mo	12 mo	849	Given	All transmission and mortality
Kisumu Breastfeeding Study (Okanda, 2014; Thomas, 2011)	Kenya (antenatal clinics)	HAART		All BF	34 wks/ 6 mo‡	6 months	12, 18, 24 mo	502	Given	All transmission and mortality
Cohan[b], 2015	Uganda, antental clinics in Tororo District	2 types of HAART		Majority BF	30 wk or less/life long	1 year	12 mo	389	Given	All transmission and mortality
Thakwalakwa[a], 2014	Malawi (Thyolo District Hospital)	Only HAART	Nutritional intervention	All BF	From first antenatal visit/ lifelong	6 mo	12 mo	248	Given	Mortality and Transmission from 6 weeks
Observational studies										
Okafor, 2014	Nigeria (Enugu State University Teaching Hospital Parklane)	HAART		BF, MF, RF	14 wk/life long	12 mo	18 mo	184	Calculated	Not clear

DREAM study (Giuliano, 2013; Palombi, 2007)	Malawi (two ante natal clinics)	HAART*		All BF	1st tremester and lifelong (CD4+<350) or week 25/ 6 mo or end BF	4.5 mo	12 and 24 mo	300	Given	All transmission and excluded death in 24h (n=3)
Alvarez-Uria[a], 2012	India (3 hospitals in Antapur)	HAART		BF and RF	From first antenatal visit / 6 mo (BF), post labour (NBF)	6 mo	12 mo	318	Given	All transmission and mortality from the 1st week
Thistle[a], 2015	Zimbabwean (Salvation Army Hospital)	HAART		All BF	Between 14 and 36 wks/ 6 mo	6 mo	12 mo	82	Calculated	All transmission and mortality
Homsy[a], 2010	Uganda (Tororo and Busia Districts)	HAART*		All BF	From first antenatal visit/ 6 mo	3-6 mo	18 mo	118	Calculated	All transmission and mortality
Peltier[a], 2009	Rwanda (four government-run health facilities)	HAART		BF and RF	28 wks / 7 mo‡	6 mo	9 mo	532	Given	All transmission and mortality from 48h
Marazzi, 2009	Mozambique	HAART		All BF	15 wks/ 6 mo‡	5 mo	12 mo	341	Given	All transmission and mortality
Kilewo, 2009	Tanzania (Dar es Salam)	HAART		All BF	34 wks/ 6 mo	6 mo	9, 12, 18 mo	441	Given	All transmission and mortality
Tonwe-Gold, 2007	Cote d'Ivoire (2 community-based antenatal clinics in Abidjan)	HAART and scARV or sdNVP*		BF and RF	24 wk/ lifelong	6 mo	12, 18 mo	261	Given	All transmission and mortality
Sagay, 2015	Nigeria	All HAART		All BF	Lifelong	1 year	18 mo	856	Given	All transmission and mortality
Ngoma, 2015	Zambia	All HAART		All BF	14 wks-lifelong	1 year	12 mo	231	Given	All transmission and mortality

[a] Studies performed in rural environment

[b] The study was designed to test the hypothesis that lopinavir/ritonavir would reduce placental malaria

†Mothers on clinical stage 4 or CD4 <200 cells/mm^3 were excluded

‡Mothers with CD4 count of <200 cells/mm3 or stage III or IV remained on HAART throughout the study, and those who subsequently met the criteria after stopping ARVs were restarted, or when CD4 cell counts ≤350 cells/mm^3 (Peltier, Marazzi)

*Mothers on HAART based on disease progression or low CD4+ count

Appendix 4 Modified NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. The item Comparability of cohorts assesses whether exposed and non-exposed individuals are matched in the design and risk for the exposure of interest is adjusted for confounders. In this study all mothers are exposed to ART, and the outcome is HIV free-survival (not relative risk or odds ratio), which is not controlled for confounders or covariates, therefore item comparability was not applied in the quality assessment.

Selection

1) Representativeness of the exposed cohort

Assesses whether the women on ART in the study are representative of women on ART in general

a) truly representative of the average women on ART in the community ✵

b) somewhat representative of the average woman on ART in the community ✵

c) selected group of users

d) no description of the derivation of the cohort

2) Ascertainment of exposure (ART)

a) secure record (e.g. clinical records) ✵

b) structured interview ✵

c) written self report

d) no description

3) Adherence to ART

a) Adherence reported in sufficient detail and adherence rates at end of study are high ✵

b) Adherence reported in sufficient detail, non-adherence <20% and unlikely to introduce bias ✵

c) Not described

4) Treatment eligibility

a) lifelong ART for all women irrespective of HIV disease progression ✵

b) ART provided for PMTCT and only for 6 months or longer if breastfeeding continues

c) Eligibility of ART on the basis of CD4 count or disease progression

5) Ascertainment of exposure (BF)

a) secure record (eg clinical records, close follow-up) ✵

b) structured interview ✵

c) written self report

d) no description

6) Duration of BF

a) Clear report of Exclusive breastfeeding up to 6 months and continue BF for 1 year ✵

b) Breastfeeding cessation at maximum 6 months

c) not described in detail

Outcome

1) Assessment of outcome

Outcome is infant death or transmission combined into HIV-free survival. This item assesses whether the information regarding infection was assessed per protocol visit and laboratory procedures, and child survival per clinical records. Where studies only report HIV transmission or only death and not HIV-free survival estimates, they do not score on this item.

a) independent assessment ✸

b) record linkage with HIV clinical programmes ✸

c) self-report

d) no description

2) Timing of outcome assessment

Infection/survival is normally assessed various times during a trial, and information should be provided on the cohorts nested in an HIV programme. Where HIV-free survival estimates are provided at more than one age point a study scores one star on this item. Studies which only report numbers of infections occurring between age points without providing a denominator at risk do not score on this item.

a) At least at two different points ✸

b) Only at one time-point

3) Outcome stratified by feeding type

a) Yes ✸

b) No

4) Was follow-up long enough for outcomes to occur

a) yes (select an adequate follow up period for outcome of interest) ✸

b) no

5) Adequacy of follow up of cohorts

a) complete follow up - all subjects accounted for ✸

b) subjects lost to follow up unlikely to introduce bias - small number lost - <20%✸

c) follow up rate < 20% and no description of those lost

d) no statement

Appendix 5. Detailed **NOS**

Studies	SELECTION																				
	Representativeness				Ascertainment ART				Adherence to ART			Treatment Eligibility			Ascertainment to BF				Duration of BF		
	Truly	*Somewhat*	*Selected*	*ND*	*Secure record*	*Structured interview*	*self report*	*ND*	*Majority*	*Some, no bias*	*ND*	*lifelong*	*6 mo*	*CD4*	*Secure record*	*Structured interview*	*self report*	*ND*	*EBF 6 mo up to 1 y*	*BF finish 6 mo*	*ND*
Giuliano **		*			*						-			-			-			-	
Marazzi **		*			*						-		-				-			-	
Ngoma ******		*			*					*		*				*			*		
Tonwe-Gold ***		*			*				*					-				-		-	
Thistle **	*				*						-		-					-		-	
Thakwalakwa **		* (govern)						-			-	*						-		-	
Cournil																					
Peltier ****		*			*				*				-	-		*				-	
Okafor ***		*				*					-	*						-			-
Shapiro ***		*				*			*				-				-			-	
Thomas ****		* (low income)			*				*				-		*					-	

Kilewo ***		*			*						-		-		*					-	
Homsy ***		*			*						-			-	*					-	
Cohan ****		*					-		*			*						-	*		
Sagay ****		*			*						-	*						-	*		
Jamieson ****		*			*					*			-			*				-	
Alvarez-Uria **		* (says)			*						-		-					-		-	
Coovadia **		*				*					-		ND					-		-	
Cournil **		*						-			43%		-			*				-	

Appendix 6: Formula used to calculate confidence intervals described by Eayres, 2008

The 100(1−α)% confidence interval limits for the proportion p are given by:

$$p_{lower} = \frac{\left(2O + z^2 - z\sqrt{z^2 + 4Oq}\right)}{2\left(n + z^2\right)}$$ **Formula 2a**

$$p_{upper} = \frac{\left(2O + z^2 + z\sqrt{z^2 + 4Oq}\right)}{2\left(n + z^2\right)}$$ **Formula 2b**

where:
q = (1−p) is the proportion without the specified characteristic;
z is the 100(1− α/2)th percentile value from the Standard Normal distribution. For example for a 95% confidence interval, α = 0.05, and z = 1.96 (i.e. the 97.5th percentile value from the Standard Normal distribution).

where:
O is the observed number of individuals in the sample/population having the specified characteristic;
n is the total number of individuals in the sample/population.